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# Selective and Validated Spectrophotometric Methods for the Determination of Nicorandil in Pharmaceutical Formulations

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Nafisur Rahman,[1] Yasmin Ahmad,[1] and Syed Najmul Hejaz Azmi[1]

[1]Department of Chemistry, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India

## ABSTRACT

Two simple and sensitive validated spectrophotometric methods have been described for the assay of nicorandil in drug formulations. Method A is based on the reaction of the drug with phloroglucinol-sulfanilic acid reagent in sulfuric acid medium to give yellow-colored product, which absorbs maximally at 425 nm. Method B uses the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) with DL- 3,4 - dihydroxyphenylalanine (DL-dopa) in the presence of nicorandil as oxidant in sulfuric acid medium to form an intensely colored product having maximum absorbance at 530 nm. Beer's law is obeyed in the concentration range 2.5 to 50.0 and 1.0 to 15.0 μg $mL^{-1}$ with methods A and B, respectively. Both methods have been successfully applied for the analysis of drug in pharmaceutical formulations. The reliability and the performance of the proposed methods are established by point and interval hypothesis and through recovery studies. The experimental true bias of all samples is smaller than ±2%.

**KEYWORDS:** nicorandil, phloroglucinol, DL-dopa, 3-methyl-2-benzothiazolinone hydrazone hydrochloride, pharmaceutical formulations, validation parameters.

## INTRODUCTION

Nicorandil is chemically known as N-[2-(nitroxy)ethyl]3-pyridine carboxamide, which belongs to the class of compounds known as potassium channel activators. Nicorandil has venodilating properties owing to the presence of nitrate group in its chemical structure. The potassium channel activation may also exert direct cytoprotective effects by augmenting normal physiological processes, which protect the heart against ischemic events.[1,2] Thus, nicorandil causes vasodilation of coronary and systematic arteries and has been investigated in the treatment of angina pectoris. Nicorandil undergoes biotransformation predominantly by denitration of nicorandil to the pharmacologically inactive alcohol metabolite, N-(2-hydroxyethyl)-nicotinamide, followed by side chain degradation to nicotinamide and related metabolites, including nicotinic acid and N-methyl-nicotinamide. The denitration occurs primarily in the liver.

The drug is officially listed in *Martindale: The Extra Pharmacopoeia*.[3] The literature revealed that the assay of the drug in pure and dosage forms is not official in any pharmacopeia and, therefore, requires much more investigation. Several analytical methods that have been reported for the estimation of nicorandil in biological fluids and/or pharmaceutical formulations include high-performance thin layer chromatography (HPTLC),[4] high-performance liquid chromatography (HPLC),[5-11] and gas chromatography coupled with mass spectrometry.[12] A review of literature revealed no UV-visible spectrophotometric method for the assay of nicorandil in pharmaceutical formulations.

This paper describes 2 simple, sensitive, selective, and economical validated visible spectrophotometric methods for the assay of nicorandil in drug formulations. The assay of drug is based on exploiting the oxidizing property of the drug due to the presence of nitrate moiety in the chemical structure of the drug. The first method is based on the reaction of the drug with phloroglucinol-sulfanilic acid reagent in sulfuric acid medium. The second method uses the oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) with DL-dopa in the presence of nicorandil as oxidant in sulfuric acid medium, resulting in the formation of an intensely colored product. The proposed methods are optimized and validated[13] as per the International Conference on Harmonisation (ICH) guidelines.

## MATERIALS AND METHODS

### *Apparatus*

A Shimadzu UV-visible spectrophotometer (model 1601, Shimadzu, Kyoto, Japan) was used for all UV-visible absorbance measurements with matched quartz cells. A water bath shaker (NSW 133, New Delhi, India) was used to control the heating temperature for the development of the color.

### *Reagents and Standards*

Phloroglucinol-sulfanilic acid reagent (0.01 M) was prepared by dissolving 0.0631 g of phloroglucinol in 2 mL of 8 M HCl and 0.0867 g of sulfanilic acid in 25 mL of doubly distilled water, separately, and then mixed and diluted to 50 mL with doubly distilled water. The solution is stable for 15 days if

**Corresponding Author:** Nafisur Rahman, Department of Chemistry, Aligarh Muslim University, Aligarh-202002, Uttar Pradesh, India. Tel: +91-571-2703515. Email: cht17nr@yahoo.co.in.

kept in the dark. For methods A and B, 12 and 15 M $H_2SO_4$ (BDH), respectively, were prepared and cooled before use.

Aqueous solutions of MBTH (0.25%; Otto Chemie, Mumbai, India) and DL-dopa (0.1%; Sigma Chemical, St Louis, MO) were freshly prepared in doubly distilled water.

Nicorandil was kindly provided by Zydus Medica, Ahmedabad, India, and was used as received. Commercial dosage forms of nicorandil such as Corflo (Wockhardt, Mumbai, India), Korandil (Sun Pharma, Mumbai, India), Nikron (Torrent, Ahmedabad, India), and Zynicor (Zydus Medica, Ahmedabad, India) were purchased locally.

The standard solution of nicorandil (0.05%; 0.5 mg $mL^{-1}$) was prepared in doubly distilled water.

### *Recommended Procedures for the Determination of Nicorandil*

*Method A*

Into a series of boiling test tubes, different volumes (0.05-1.0 mL) of 0.05% nicorandil were pipetted. To each test tube, 2.5 mL of 12 M sulfuric acid and 2.5 mL of 0.01 M phloroglucinol-sulfanilic acid reagent were added, mixed well, and heated on a water bath at 100°C ± 1°C for 32 minutes. The tubes were cooled at room temperature (25°C ± 1°C), and then the contents of the tubes were transferred to 10-mL volumetric flasks and diluted to volume with doubly distilled water. The absorbance was measured at 425 nm against a reagent blank treated similarly except without drug within the stability period of 1 hour. The concentration of nicorandil was calculated either from calibration curve or from regression equation.

*Method B*

Aliquots of 0.05 to 0.75 mL of 0.05% nicorandil solution corresponding to 25.0 to 375.0 µg were pipetted into a series of boiling test tubes. Eight milliliters of 15 M $H_2SO_4$, 2.5 mL of 0.25% MBTH, and 0.5 mL of 0.1% DL-dopa were added to each tube. The contents of each tube were mixed well and heated in a water bath at 100°C ± 1°C for 12 minutes. After cooling at room temperature, the contents of the tubes were transferred to 25-mL standard flasks and diluted to volume with doubly distilled water. The color was stable up to 4 hours. The absorbance was measured at 530 nm against the reagent blank treated similarly except without drug. A calibration graph was drawn and the corresponding regression equation was computed to obtain the concentration of nicorandil.

*Preparation of Denitrated Nicorandil*

Nicorandil (500 mg) was hydrolyzed with 4N HCl and 4N NaOH at 100°C ± 1°C for 30 minutes. Preparative thin layer chromatography was applied using silica gel G plate and chloroform:methanol:ethyl acetate (4:1:5 vol/vol/vol) as mobile phase. The band corresponding to the degradation product was located under UV lamp at 254 nm. The band was scrapped and extracted with chloroform. The solvent was removed and pure degradation product was obtained.

*Procedure for the Assay of Nicorandil in Pharmaceutical Formulations*

To minimize a possible variation in the composition of the tablets, the mixed contents of 20 tablets were weighed and grounded; then the powder equivalent to 50 mg nicorandil was stirred well with dichloromethane and filtered through Whatman No. 42 filter paper (Whatman International Limited, Kent, UK). The residue was washed with dichloromethane for complete recovery of the drug. The filtrate was evaporated to dryness under vacuum and the left drug was taken up with doubly distilled water and transferred to a 100-mL standard flask and completed to volume with doubly distilled water. The percentage recovery of the drug was calculated from the corresponding linear regression equations.

*Procedure for Reference Method*

Into a series of 25-mL standard volumetric flasks, different volumes containing 0.4 to 10.0 µg $mL^{-1}$ of drug (0.01%; 0.1 mg $mL^{-1}$) solution were pipetted and diluted to volume with doubly distilled water. The absorbance was measured against a solvent blank at 262.5 nm. The amount of the drug in a given sample was computed from the calibration equation.

## RESULTS AND DISCUSSION

The electrophilic substitution reaction of phloroglucinol with $N_2O_5$ in sulfuric acid medium has been studied,[14] resulting in the formation of yellow-colored species of 1,3,5-trihydroxy-2,4,6-trinitrobenzene. The denitration of nicorandil occurs in acidic medium.[4] Therefore, the reaction of nicorandil with phloroglucinol in sulfuric acid medium leads to the formation of 1,3,5-trihydroxy-2,4,6-trinitrobenzene, which absorbs maximally at 425 nm (Figure 1A). According to TLC experiments, the isolated denitrated nicorandil is more polar than nicorandil due to the denitration process. The denitrated nicorandil did not interfere with phloroglucinol, thus confirming the reaction of nicorandil with phloroglucinol. The nitrite, which is formed due to denitration of nicorandil, interferes positively and must be destroyed by sulfanilic acid. Therefore, based on the literature background and experimental findings, the reaction mechanism was proposed and is given in Scheme 1.

Nicorandil, being an oxidant, oxidizes MBTH (1) in acidic medium resulting in the formation of an electrophilic inter-

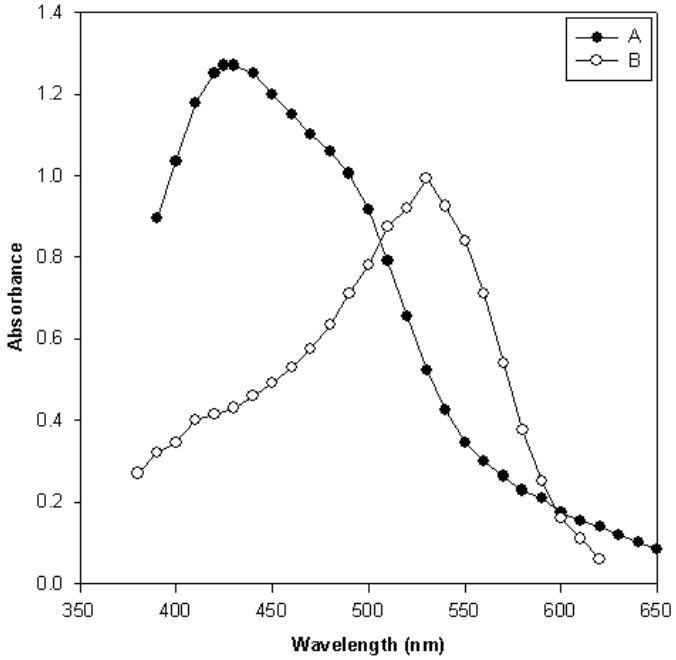



**Figure 1.** Absorption spectra of colored products of nicorandil (A) 0.9 mL of 0.05% nicorandil + 2.5 mL of 12 M $H_2SO_4$ + 2.2 mL of 0.01 M phloroglucinol-sulfanilic acid and (B) 0.7 mL of 0.05% nicorandil + 8.0 mL of 15 M $H_2SO_4$ + 2.6 mL of 0.25% MBTH + 0.6 mL of 0.1% DL-dopa.

mediate (2), which is an active coupling species.[15,16] The intermediate of MBTH undergoes electrophilic substitution with the phenolic moiety of DL-dopa to form a colored product showing an absorption peak at 530 nm (Figure 1B). The proposed reaction mechanism is presented in Scheme 2.

### *Optimization of Variables*

The spectrophotometric properties of the colored species formed with methods A and B were extensively studied. The optimum conditions for the assay procedures (methods A and B) have been established by studying the reactions as a function of heating time, concentration of reagents, sulfanilic acid, and stability of the colored species.

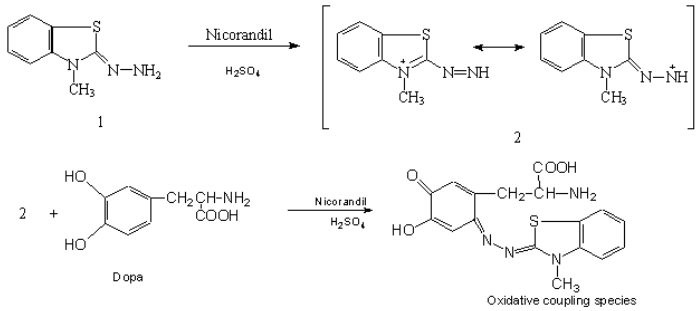
### *Method A*

#### *Effect of Heating Time*

To study the effect of heating time for maximum color development, 0.9 mL of 0.05% nicorandil was mixed with 2.5 mL of 0.01 M phloroglucinol-sulfanilic acid reagent and 2.5 mL of 12 M $H_2SO_4$. The contents of the mixture were heated up to 35 minutes in a water bath at 100°C ± 1°C. It is apparent from investigations that the maximum intensity of color was attained after 30 minutes of heating and remained constant up to 35 minutes. Therefore, the optimum heating time was fixed at 32 minutes throughout the experiment.

**Scheme 1.**

**Scheme 2.**




#### *Effect of the Concentration of Sulfuric Acid*

The influence of the volume of 12 M $H_2SO_4$ was observed during the formation of yellow-colored product. To study this, an aliquot of drug containing 450 µg was pipetted followed by varying volumes (0.1-2.5 mL) of 12 M $H_2SO_4$ and 2.2 mL of 0.01 M phloroglucinol-sulfanilic acid. It is evident from Figure 2A that the highest absorbance was attained with 2.2 mL of 12 M $H_2SO_4$; above this volume the absorbance remained unchanged. Therefore, 2.5 mL of 12 M $H_2SO_4$ was used in all further measurements.

#### *Effect of the Concentration of Phloroglucinol-Sulfanilic Acid*

To investigate the effect of volume of 0.01 M phloroglucinol-sulfanilic acid reagent for color development, different volumes (0.3-2.5 mL) were mixed with 0.9 mL of 0.05% nicorandil and 2.5 mL of 12 M $H_2SO_4$. The results are presented in Figure 2B, which reveals that the addition of 2.2 mL gave the highest absorbance, which remained constant up to 2.5 mL. Therefore, 2.2 mL of the reagent was taken for the determination of the drug throughout the experiment.

### *Method B*

#### *Effect of Heating Time*

The optimum heating time for the reaction to complete was evaluated by heating a mixture containing 0.7 mL of 0.05%

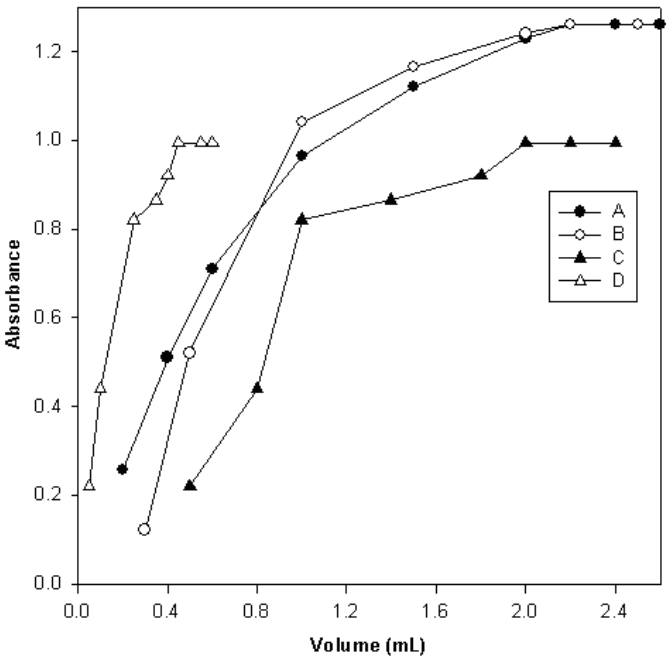



**Figure 2.** Effect of the volume of (A) 12 M $H_2SO_4$ and (B) 0.01 M phloroglucinol-sulfanilic acid reagent (method A); (C) 0.25% MBTH and (D) 0.1% DL-dopa (method B).

nicorandil, 8 mL of 15 M $H_2SO_4$, 2.6 mL of 0.25% MBTH, and 0.6 mL of 0.1% DL-dopa in a water bath at 100°C ± 1°C. The intensity of the colored product reached maximum at 10 minutes and remained constant up to 15 minutes. Therefore, the optimum heating was fixed at 12 minutes.

*Effect of the Concentration of Sulfuric Acid*

The effect of the volume of 15 M sulfuric acid on color development of the product was investigated by taking 0.7 mL of 0.05% nicorandil with varying volumes (0.5-9 mL) of 15 M $H_2SO_4$, 2.6 mL of 0.25% MBTH, and 0.6 mL of 0.1% DL-dopa into a series of test tubes. The reaction mixture of each test tube was heated in a water bath for 12 minutes and transferred to 25-mL standard flasks and diluted to volume with doubly distilled water. The highest absorbance was obtained with 7 mL of 15 M $H_2SO_4$; above this volume, no change in absorbance was recorded. Therefore, 8 mL of 15 M $H_2SO_4$ was used in all determinations.

*Effect of MBTH Concentration*

The effect of volume of 0.25% MBTH on the color development was investigated over the range 0.5-3.0 mL. The results are presented in Figure 2C, which showed that 2.4 mL was adequate to give maximum intensity of the color. Hence, 2.6 mL of 0.25% MBTH was used as an optimum value for color development.

*Effect of DL-dopa Concentration*

The effect of DL-dopa concentration was studied by adding different volumes (0.05-0.7 mL) of 0.1% DL-dopa, 8 mL of 15 M $H_2SO_4$, and 2.6 mL of 0.25% MBTH to a constant amount of 350 µg per 25 mL of nicorandil. It was found that the maximum intensity of the violet color was reached with 0.45 mL of reagent and remained so with higher volumes (Figure 2D). Therefore, 0.6 mL of the reagent was used throughout the experiment.

*Analytical Data and Calibration Graphs*

Under the optimized experimental conditions, straight line calibration graphs were obtained over the calibration ranges 2.5-50.0 and 1.0-15.0 µg $mL^{-1}$ of nicorandil with molar absorptivities of $5.914 \times 10^3$ and $1.500 \times 10^4$ L $mol^{-1}$ $cm^{-1}$ with methods of A and B, respectively. The linear regression equations for both the methods have been evaluated by least square treatment of the calibration data (n = 9). Table 1 summarizes Beer's law limit, linear regression equation, correlation coefficient, confidence limits, and standard deviations for slope and intercept at 95% confidence level, variance, and detection limits for methods A and B. In each method, the correlation coefficient was high, indicating the excellent linearity of both the calibration graphs. The low values of confidence interval at 95% confidence level for slope and intercept of the regression lines pointed toward high reproducibility of the proposed methods. In order to verify that the developed methods are free from procedural errors, the experimental intercepts, *a*, of lines of regression were tested for significance of the deviation from the expected value zero.[17,18] For this justification, the values calculated for *t* from the relation, $t = \frac{a}{S_a}$ were found to be 0.058 and 0.222 for methods A and B, respectively, which did not exceed the 95% criterion, 2.365 (ν = 7). It is concluded that the intercepts for methods A and B are not significantly different from zero. Thus, the proposed methods (A and B) are free from constant errors independent of the concentration of nicorandil. The detection limit (DL) at 95% confidence level was established using the relation[19,20]:

$$DL = \frac{t}{b}\sqrt{s_0^2 \times \frac{n-2}{n-1}}, \quad (1)$$

where n is the number of standard samples (n = 9), *t* is the value of Student *t* test for n – 2 degrees of freedom at 95% confidence level and $S_0^2$ = variance. Both the DL and the slope of the calibration graphs indicated good sensitivity. The variance was calculated using the equation[21]:

$$S_0^2 = \frac{\sum (A_{expr} - A_{calc})^2}{n-2} \quad (2)$$

and was found to be very low for methods A and B, indicating negligible scattering of the experimental data points around the line of regression.

**Table 1.** Optical and Regression Characteristics of the Proposed Methods

| Parameters | Method A | Method B |
|---|---|---|
| $\lambda_{max}$ (nm) | 425 | 530 |
| Beer's law limit (μg mL$^{-1}$) | 2.5 - 50.0 | 1.0 - 15.0 |
| Molar absorptivity (L mol$^{-1}$ cm$^{-1}$) | $5.914 \times 10^3$ | $1.500 \times 10^4$ |
| Linear regression equation* | $A = 1.164 \times 10^{-4} + 2.805 \times 10^{-2}$ C | $A = 7.619 \times 10^{-5} + 7.099 \times 10^{-2}$ C |
| Intercept (a) | $1.164 \times 10^{-4}$ | $7.619 \times 10^{-5}$ |
| $S_a$ | $2.014 \times 10^{-3}$ | $3.431 \times 10^{-4}$ |
| $tS_a$† | $4.762 \times 10^{-3}$ | $8.114 \times 10^{-4}$ |
| Slope (b) | $2.805 \times 10^{-2}$ | $7.099 \times 10^{-2}$ |
| $S_b$ | $6.751 \times 10^{-5}$ | $3.671 \times 10^{-5}$ |
| $tS_b$‡ | $1.600 \times 10^{-4}$ | $8.662 \times 10^{-5}$ |
| Correlation coefficient (r) | 0.9999 | 0.9999 |
| Variance ($S_0^2$) | $1.142 \times 10^{-5}$ | $2.830 \times 10^{-7}$ |
| Detection limit (μg mL$^{-1}$) | 0.267 | 0.017 |

*With respect to A = a + bC, where C is the concentration (μg mL$^{-1}$) and A is absorbance.
†Confidence interval of the intercept at 95% confidence level.
‡Confidence interval of the slope at 95% confidence level.

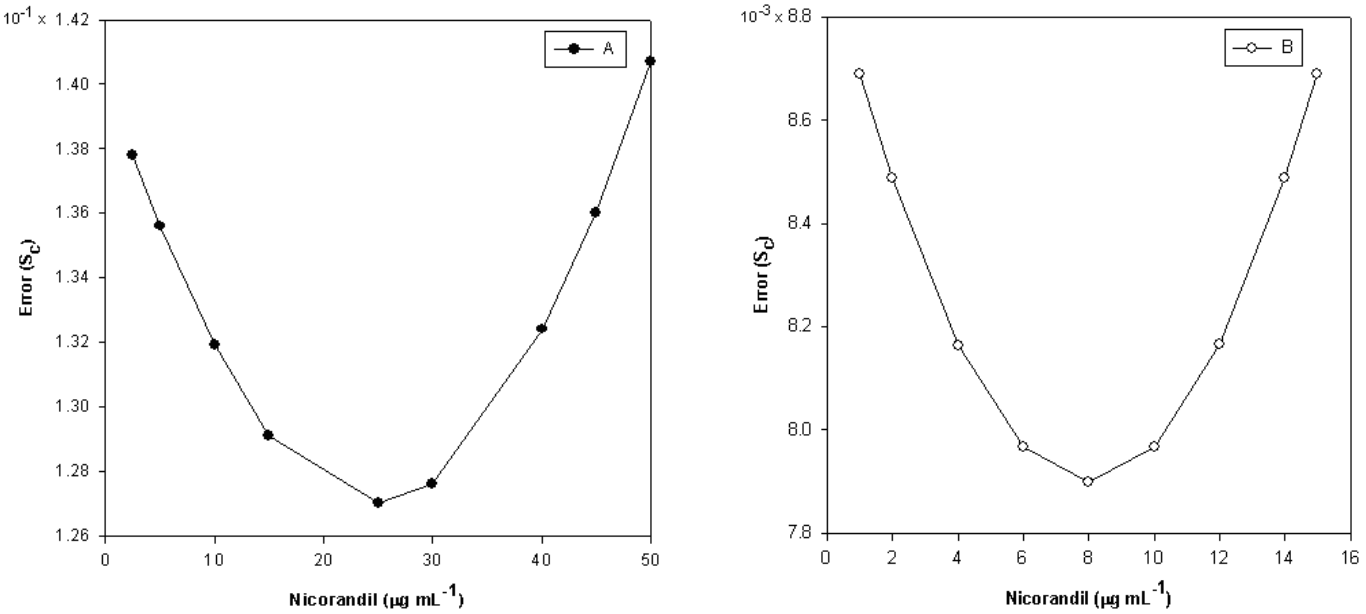



**Figure 3.** Error in the determination of the concentration of nicorandil obtained by statistical analysis of standard calibration data for (A) method A and (B) method B.

The absolute error, $S_c$, was calculated in the determination of nicorandil for methods A and B by means of statistical analysis of calibration data using the relation[22]:

$$S_c = \sqrt{\frac{\sum (A - \bar{A})^2}{n-2}} \sqrt{\frac{1}{n} + \frac{(C - \bar{C})^2}{\sum (C - \bar{C})^2}} \quad (3)$$

where $\bar{C}$ and $\bar{A}$ are the average concentration and absorbance values, respectively, for n standard solutions. Figures 3A and 3B show the graph of $S_c$ versus the final concentration of nicorandil. The error has reached minimum when the actual absorbance is equal to the average absorbance corresponding to ~24.7 and 8.0 μg mL$^{-1}$ for methods A and B, respectively. The confidence limits for unknown concentrations for nicorandil can be determined using the relation $C_i \pm tS_c$ at a selected confidence level and n – 2 degrees of freedom. The results are shown in Figures 4A and 4B in the form of percentage uncertainty,[23] $\frac{tS_c}{C_i} \times 100$ against the concentration of nicorandil at 95% confidence level. Thus, the relative uncertainty can be estimated directly on the concentration level tested.

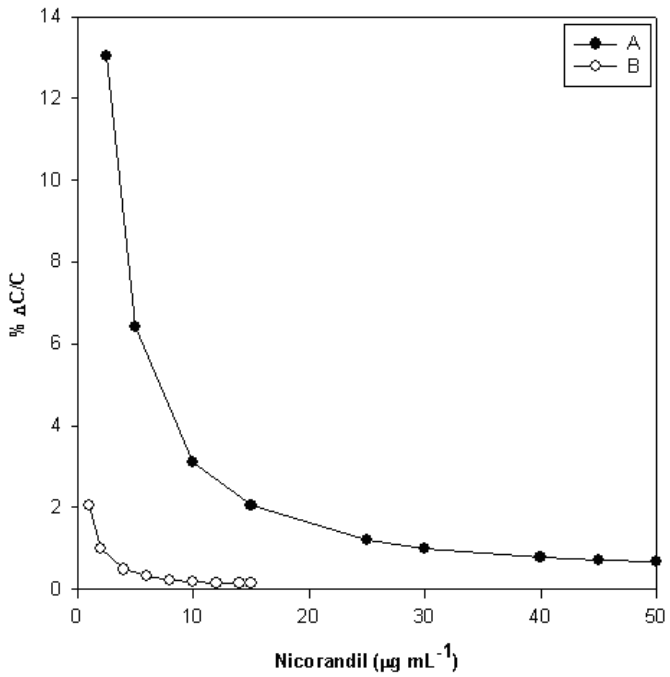



**Figure 4.** Variation of the confidence limits for (A) method A and (B) method B at 95% confidence level and n – 2 degrees of freedom.

### *Solution Stability*

The stability of nicorandil solution was examined by recording absorption spectra of the solution for several days and by TLC studies, too. The band corresponding to degradation product was not observed under UV lamp at 254 nm, and there was also no change in the spectra for at least 4 days, when the solution was stored at room temperature.

### *Specificity*

The specificity of the proposed methods was evaluated by determining the nicorandil concentration in the presence of varying amounts of denitrated nicorandil. It was found that the degradation product did not react with either reagent.

### *Ruggedness*

The ruggedness of the method relative to each operational parameter was challenged. The operational parameters investigated were:

*For Method A*

- volume of 12 M $H_2SO_4$ (±0.3 mL)
- volume of 0.01 M phloroglucinol-sulfanilic acid reagent (±0.3 mL)
- heating time (±2.0 minutes)
- cooling temperature (±5°C)

*For Method B*

- volume of 15 M $H_2SO_4$ (±1.0 mL)
- volume of 0.1% DL-dopa (± 0.15 mL)
- volume of 0.25% MBTH (±0.2 mL)
- heating time (±2.0 minutes).

The ruggedness of the proposed methods relative to each operational parameter was examined by analyzing the nicorandil tablets under variable experimental conditions. For this, a sample solution containing 10 μg $mL^{-1}$ (Korandil-10) was assayed 5 times using both the methods. The results showed a mean value of 9.99 ± 0.06 μg $mL^{-1}$ and 10.02 ± 0.05 μg $mL^{-1}$ with relative standard deviations of 0.60% and 0.54% for methods A and B, respectively. These results indicated the ruggedness of the proposed methods.

### *Robustness*

The method robustness was evaluated by a second analyst using a different instrument and freshly prepared standard and sample solutions. The analysis of the nicorandil tablets was performed 5 times at 1 concentration level by the robustness chemist and developing chemist following the recommended procedures. The results agreed well within the acceptable limits and no degradate was found to interfere with the determination process. These results demonstrated acceptable method robustness.

### *Precision and Accuracy*

The short-term precision (intraday precision) of methods A and B were evaluated by measuring 5 independent samples of nicorandil in pure form at 3 different concentration levels (10.0, 30.0, 50.0 μg $mL^{-1}$ for method A and 4.0, 10.0, 15.0 μg $mL^{-1}$ for method B), and in pharmaceutical formulations at 1 concentration level (10 μg $mL^{-1}$). The standard deviations and relative standard deviations for methods A and B were in the range of 0.04 to 0.42 μg $mL^{-1}$, 0.16% to 0.92%, and 0.05 to 0.12 μg $mL^{-1}$, and 0.50% to 1.13%, respectively. In the same manner, the assay for daily precision (interday precision) at each concentration level was repeated for 5 consecutive days. The standard deviations and relative standard deviations for methods A and B were found to vary over the range 0.05 to 0.56 μg $mL^{-1}$, 0.19% to 1.12%, and 0.05 to 0.15 μg $mL^{-1}$, and 0.55% to 1.29%, respectively. The values of standard deviation and relative standard deviation can be considered to be very satisfactory, and thus the proposed methods (A and B) are very effective for the determination of nicorandil in pure form and pharmaceutical formulations.

The reliability and accuracy of the proposed methods were further ascertained through recovery studies using the stan-

**Table 2.** Point Hypothesis Test: Comparison of the Proposed Methods With the Reference Method at 95% Confidence Level

| Pharmaceutical Formulations | Method A | | | | Method B | | | | Reference method | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Recovery % | RSD* % | *t*-value† | F-value† | Recovery % | RSD* % | *t*-value† | F-value† | Recovery % | RSD* % |
| Corflo-10 (Wockhardt) | 99.99 | 0.48 | 0.40 | 1.01 | 100.06 | 0.50 | 0.17 | 1.11 | 100.11 | 0.48 |
| Korandil-10 (Sun Pharm.) | 99.92 | 0.60 | 0.59 | 1.90 | 100.17 | 0.54 | 0.19 | 1.55 | 100.11 | 0.43 |
| Nikron-10 (Torrent) | 100.07 | 0.59 | 0.21 | 1.05 | 99.95 | 0.53 | 0.12 | 1.15 | 99.99 | 0.57 |
| Zynicor-10 (Zydus Medica) | 100.14 | 0.71 | 0.20 | 1.68 | 100.12 | 0.66 | 0.15 | 1.43 | 100.06 | 0.55 |

*RSD indicates Relative standard deviation. Mean of 5 independent analyses.

†Theoretical *t*-value ($\nu = 8$) and F-value ($\nu = 4, 4$) at 95% confidence level are 2.306 and 6.39, respectively.

**Table 3.** Interval Hypothesis Test: Comparison of the Proposed Methods With the Reference Method at 95% Confidence Level

| Pharmaceutical Formulations | Method A | | Method B | |
|---|---|---|---|---|
| | Lower Limit* ($\theta_L$) | Upper Limit* ($\theta_U$) | Lower Limit* ($\theta_L$) | Upper Limit* ($\theta_U$) |
| Corflo-10 (Wockhardt) | 0.990 | 1.007 | 0.991 | 1.008 |
| Korandil-10 (Sun Pharm) | 0.989 | 1.007 | 0.992 | 1.009 |
| Nikron-10 (Torrent) | 0.991 | 1.011 | 0.990 | 1.009 |
| Zynicor-10 (Zydus Medica) | 0.990 | 1.012 | 0.990 | 1.011 |

*In pharmaceutical analysis, a bias, based on recovery experiments, of ± 2% ($\theta_L$=0.98 and $\theta_U$=1.02) is acceptable.

dard addition method. For this purpose, a fixed amount of nicorandil from preanalyzed tablets was taken and an amount of the pure drug (standard) at 2 different concentration levels was added and the total amount was estimated by the proposed methods A and B. Each level was repeated 5 times using 4 different commercial pharmaceutical formulations. The results obtained for methods A and B through the standard addition method showed that the mean recoveries and relative standard deviations were in the range of 99.93% to 100.53%, 0.14% to 0.87%, 99.95% to 100.97%, and 0.45% to 1.15%, respectively, which can be considered to be very satisfactory. No interference from commonly encountered tablet excipients such as talc, starch, gum acacia, lactose, sodium alginate, and magnesium stearate was observed in the determination. Methods A and B were successfully applied to the determination of nicorandil in pharmaceutical formulations. The results of the proposed methods (A and B) were compared with those of the reference method using point hypothesis tests. Table 2 shows that the calculated *t* and F values are less than theoretical ones,[24] confirming accuracy and precision are within the acceptable limits and indicating no significant difference between the performance of the proposed methods and the reference method at 95% confidence level. The interval hypothesis tests[25] have also been performed to compare results of the proposed methods (A and B) with those of the reference method at 95% confidence level (Table 3). The usual practice in attempting the point and interval hypothesis tests in hospitals and laboratories is to make sure that the standard deviation values of each assay are within the acceptable limits. Once this is established, the actual analytical error is usually ignored and not reported along with the concentration itself.[26] Hence, it was decided that a bias of ±2% is acceptable. Therefore, the limit of acceptance interval is within $\theta_L = 0.98$ and $\theta_U = 1.02$. It is clear from Table 3 that the true bias of all samples is smaller than ±2%. The interval hypothesis tests draw the same conclusion as the point hypothesis tests. Thus, the proposed methods shown here are accurate, precise, and validated.

## CONCLUSION

The proposed methods are sensitive and selective owing to the oxidizing nature of the drug, which preferentially interacts with reagents described in the subsection Reagents and Standards, but its major metabolite (denitrated nicorandil) did not give the positive results with the reagents used. Point and interval hypothesis tests and recovery data clearly proved that the proposed methods have acceptable precision, accuracy, and linearity.

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